PDE8: a Novel Target in Human Airway Smooth Muscle Cells

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Abstract

The ubiquitous second messenger 3',5'-cyclic adenosine monophosphate (cAMP) acts as a key player in the signaling cascades which control many physiological and pathophysiological processes in the lung. The intracellular concentrations of cAMP are determined by its production, which is regulated by G-protein coupled receptors (GPCRs) and adenylyl cyclases (ACs), and its degradation, which is modulated by phosphodiesterases (PDEs). In the current issue of the American Journal of Respiratory Cell and Molecular Biology, Johnstone et al. demonstrate for the first time the transcript, protein and functional presence of PDE8 in human ASM cells and report on a functional β_2-AR-AC6-PDE8 signalosome expressed in caveolae. In view of the therapeutic value that PDE inhibitors may have, both the scientific and therapeutic value of this novel finding are discussed.

The ubiquitous second messenger 3',5'-cyclic adenosine monophosphate (cAMP) acts as a key player in the signaling cascades which control many physiological and pathophysiological processes in the lung. The intracellular concentrations of cAMP are determined by its production, which is regulated by G-protein coupled receptors (GPCRs) and adenylyl cyclases (ACs), and its degradation, which is modulated by phosphodiesterases (PDEs), which comprise 11 family members (from 1 to 11) and at least 21 isoforms with different splice variants (Page and Spina, 2012), are able to hydrolyze cyclic nucleotides (cAMP and cGMP) to their inactivate 5' monophosphates within subcellular microdomains, thereby modulating cyclic nucleotides signaling pathways. The cAMP-specific PDEs are PDE4, PDE7 and PDE8, while PDE5, PDE6 and PDE9 are cGMP-specific. The remaining family members can degrade both cyclic nucleotides, although they prefer one or the other to varying degrees.

PDE4 is the most extensively studied cAMP-specific PDE, which is highly expressed in airway smooth muscle (ASM) cells. Many studies have provided important information regarding the functions of PDE4 in ASM cells, especially regulating cell proliferation, contraction and migration (Kolosionek et al., 2009; Lin et al., 2016; Méhats et al., 2003; Trian et al., 2011). A-kinase anchoring proteins (AKAPs) also contribute to spatial and temporal cAMP dynamics by binding directly to protein kinase A (PKA) and its target proteins, thus physically tethering these multi-protein complexes to specific locations in the cells (Poppinga et al., 2014). It has been demonstrated that gravin (AKAP250) is able to tether PKA and PDE4D to β_2-adrenoceptor (β_2-AR) at the plasma membrane in HEK cells, highlighting the importance of compartmentalized cAMP signaling (Willoughby et al., 2006). As another cAMP-specific enzyme, PDE7 inhibition is also believed to induce a variety of cellular effects due to increased cAMP accumulation. BRL50481, a PDE7 selective inhibitor, can relax the airways after histamine-induced contractions in ovalbumin-sensitized guinea pigs model (Mokry et al., 2013). Moreover, the inhibition of PDE7 augments the inhibitory effect of other cAMP-elevating drugs on the proinflammatory cells without having such effect itself (Smith et al., 2004). However, surprisingly, the role and location of PDE8, a less widely expressed PDE member which has greater cAMP affinity compared to PDE4, has not been studied in ASM cells yet.

In the current issue of the American Journal of Respiratory Cell and Molecular Biology, Johnstone et al. (Timothy B. Johnstone et al., n.d.) demonstrate for the first time the transcript, protein and functional presence of PDE8 in human ASM cells and report on a functional β_2-AR-AC6-PDE8 signalosome expressed in caveolae. To study the functional role of PDE8, dipyridamole, a semiselective PDE5/8 inhibitor, was used to enhance cAMP accumulation after forskolin stimulation in human ASM cells overexpressing either AC2 or AC6. Then authors found that increased cAMP levels were restricted to ASM cells overexpressing AC6. These findings were further confirmed using shRNA knockdown of PDE8A, indicating that PDE8A was able to hydrolyze cAMP specifically when induced by AC6, with little interference on AC2 induced cAMP generation. Most intriguingly, this study revealed that PDE8 inhibition selectively increased cAMP levels which was generated by β_2-AR, but had no effect

when EP_2 or EP_4 receptors were activated. Thus, PDE8 inhibition, together with β_2-AR stimulation, reduced serum-induced human ASM cell proliferation, while PDE8 inhibition had no such effect on PGE2. Modulation of PDE8 activity in human ASM cells possibly enhances the effect of β_2-AR on bronchodilation as well, but this needs to be further established in future studies.

Nowadays, several cAMP biosensors are designed to visualize cAMP fluctuations in living cells with high temporal and spatial resolution (Sprenger and Nikolaev, 2013). After almost two decades, the fluorescence resonance energy transfer (FRET)-based cAMP sensors are well developed and widely used in studying cAMP dynamics to address such questions. In the present study, Johnstone et al. used another novel genetically encoded cAMP biosensor named cAMP Difference Detector in situ (cADDis) to monitor cAMP dynamics by testing in a much easier standard fluorescent plate reader (Timothy B. Johnstone et al., n.d.). Comparing to the classical two fluorophores FRET-based cAMP biosensor, cADDis is composed of one circularly permuted green fluorescent protein and a cAMP binding domain of exchange protein directly activated by cAMP (EPAC) 2, which makes it possible to be paired with other colored sensors for multiplex recordings, such as red Ca2+ sensors (Tewson et al., 2016). Uisng this sensor, the authors were able to show that the PDE8 selective inhibitor PF-04957325 had strikingly large effects on cAMP in ASM, indicating PDE8 activity is at least as important as PDE4 in modulating cAMP dynamics in human ASM cells. Possibly, PDE8 has a therapeutic value therefore in respiratory diseases, such as asthma and chronic obstructive pulmonary diseases (COPD).

Several intriguing questions arise from this study that require further investigation. cAMP has been shown to regulate ASM contractile state, inflammatory cytokines and chemokines secretion, cell proliferation and migration (Billington et al., 2013). Therefore, it is important to examine the effect of PDE8 inhibition on these responses in further detail in both in vitro and in vivo experimental models. Moreover, AKAPs, as one of the most important elements in cAMP compartments, play a vital role in modulating cAMP spatial and temporal dynamics. Thus, it is rational to explore the role of different AKAPs members in controlling PDE8 activity and localization. The present study shows the co-localization of PDE8 with β_2-AR-AC6 in caveolae.

In conclusion, the study of Johnstone et al. (Timothy B. Johnstone et al., n.d.) reveals for the first time the selective role of PDE8 in limiting β_2-AR-AC6 mediated cAMP signaling in human ASM cells, and motivates further exploration on the functional outcomes of PDE8 inhibition. As one of the most predominant cAMP hydrolyzing PDEs in human ASM cells, PDE8 seems to be a therapeutic strategy worth pursuing in respiratory diseases.

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